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Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Enclosed is a patent application (including formal papers) as follows:

Applicants: Shu-Ching Cheng, Ming-Shian Wu, Herbert Bradfield Cunningham, Ching Huang, Eugene Fan and Michael Willrodt

Title: **METHODS OF USE OF ONE STEP IMMUNOCHROMATOGRAPHIC
DEVICE FOR STREPTOCOCCUS A ANTIGEN**

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being deposited with the United States Postal Service on the date shown below with sufficient postage as Express Mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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If this application is found otherwise to be incomplete, or if at any time appears that a telephone conference with counsel would helpfully advance prosecution, please telephone the undersigned in La Jolla, California (619) 552-8400.

Please charge our Deposit Account No. 12-2475 for the appropriate amount.

Kindly acknowledge receipt of the foregoing application by returning the self-addressed postcard.

Respectfully submitted,

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Enclosures

DESCRIPTION**METHODS OF USE OF ONE STEP IMMUNOCHROMATOGRAPHIC DEVICE
FOR STREPTOCOCCUS A ANTIGEN**Background of the Invention

5 This invention relates to immunological methods and
devices for detecting analytes in biological samples.

 Numerous approaches have been developed for
detection of a given analyte in a biological sample.
Typical of these methods are the so called "lateral
10 flow" and "flow-through" devices and methods. The flow-
through device generally uses a porous material with a
reagent-containing matrix layered thereon or
incorporated therein. Test sample is applied to and
flows through the porous material, and analyte in the
15 sample reacts with the reagent(s) to produce a
detectable signal on the porous material. These devices
are generally encased in a plastic housing or casing
with calibrations to aid in the detection of the
particular analyte.

20 Lateral flow assays also utilize a porous membrane

5 for performing analyte detection. Instead of drawing
the sample through the membrane perpendicularly, the
sample is permitted to flow laterally from an
application zone to a reaction zone on the membrane
surface. The capture reagent is present in the reaction
10 zone, and the captured analyte can be detected by a
variety of protocols, including direct visualization of
visible moieties associated with the captured analyte.

One-step lateral flow assays permit a user to add a
sample to a sample application region and obtain a
15 positive or negative signal signaling the presence or
absence of the test analyte in the sample.

One-step lateral flow devices contain a sample
application region to which the liquid sample is
applied. The sample application region is in lateral
20 flow contact with the porous carrier material of the
analyte detection region. During lateral flow, the
sample is brought into contact with a mobile indicator
reagent in a discrete zone of the analyte detection
region. The indicator reagent contains both a binding
25 moiety which specifically binds to the target analyte

5 and an indicator moiety, which is most often a
chromophore label. Target analyte molecules moving in
the lateral flow bind to the indicator reagent and are
ultimately immobilized in the capture zone, usually by
binding to a second reagent which binds specifically to
10 the analyte or to the analyte-indicator reagent complex,
giving rise to a positive test signal. Additional
signals may include a negative reaction indicator, a
test complete indicator, and a positive control
indicator.

15 One-step immunochromatographic devices containing
the indicator reagent in a discrete zone of the lateral
flow porous material, e.g., at a discrete site on the
test strip, have been described.

20 Lateral flow assays also utilize a porous membrane
for performing analyte detection. Instead of drawing
the sample through the membrane perpendicularly,
however, the sample is permitted to flow laterally from
an application zone to a reaction zone on the membrane
surface. The capture reagent is present in the reaction
25 zone, and the captured analyte can be detected by a

5 variety of protocols, including direct visualization of
visible moieties associated with the captured analyte.

For example, Hochstrasser, U.S. Patent 4,059,407,
discloses a dipstick device which can be immersed in a
biological fluid to semi-quantitate analyte in the
10 fluid. Semi-quantitation of the analyte is accomplished
by using a series of reagent-containing pads wherein
each pad in the series will produce a detectable color
(i.e., a positive result) in the presence of an
increasing amount of analyte.

15 In U.S. Patent Nos. 4,094,647, 4,235,601 and
4,361,537, Deutsch et al. describe immunoassays of
certain liquid samples deposited on a chromatographic
test strip device. The device comprises a material
capable of transporting a solution by capillary action,
20 i.e., wicking. Different areas or zones in the strip
contain the reagents needed to produce a detectable
signal as the analyte is transported to or through such
zones. The device is suited for both chemical assays
and binding assays which are typified by the binding
25 reaction between an antigen and its complementary

5 antibody.

Many variations on the Deutsch et al. device have also been disclosed. For example, Grubb et al., U.S. Patent No. 4,168,146 describes the use of a porous test strip material to which is covalently bound an antigen-specific antibody. In performance of an assay, the test strip is immersed in a solution suspected of containing an antigen, and capillary migration of the solution up the test strip is allowed to occur. As the antigen moves up the test strip it binds to the immobilized antigen-specific antibody. The presence of antigen is then determined by wetting the strip with a second antigen-specific antibody to which a fluorescent or enzyme label is covalently bound. Quantitative testing can be achieved by measuring the length of the strip that contains bound antigen.

In addition, European Publication No. 323,605 discloses an assay device using chromatographic material wherein the test sample can travel from one end to the other by capillary action. The chromatographic material contains an immobilized capture reagent capable of

5 binding to the analyte. The application pad receives
the test sample and contains a diffusive indicator
reagent capable of migrating from the application pad to
the chromatographic material. The indicator reagent is
capable of binding to the analyte or the capture
10 reagent. The binding of the indicator reagent results
in a detectable signal.

Other disclosures of lateral flow assays have also
appeared. For example, U.S. Patent No. 4,861,711 de-
scribes a lateral flow assay wherein all components
15 needed for the detection of an analyte are embedded in a
single sheet. The lateral flow is referred to as
chromatographic behavior. This patent discloses the use
of enzyme antibody conjugates and substrates, each
separately held in absorbent pads. European Patent
20 Application 306,772 describes a lateral flow device
which comprises a chromatographic medium wherein the
zone for application of sample and the reaction zone
with an immobilized reagent capable of binding the
analyte or a label-specific binding material are
25 separated. British Application No. 2,204,398 describes

5 the use of a lateral flow device for testing hCG in
urine samples, where sample applied to the device picks
up labeled reagent and permeates into a detection zone.
Labels include gold sols and colored particles.

10 For instance, European Publication No. 323,605
discloses an assay device using chromatographic material
wherein the test sample can travel from one end to the
other by capillary action. The chromatographic material
contains an immobilized capture reagent capable of
binding to the analyte. The application pad which
15 receives the test sample also contains a diffusible
indicator reagent capable of migrating from the
application pad to the chromatographic material. The
indicator reagent is capable of binding to the analyte.
The binding of the indicator reagent-analyte complex
20 results in a detectable signal at the capture situs.

PCT application No. WO 94/06013 also describes a
lateral flow assay in which the indicator reagent has
been placed in a separate labeling reagent region or pad
(referred to as "the third liquid permeable material").

25 The sample is added to a separate sample application

5 pad, passes through a second permeable material, and
mobilizes the indicator reagent located in the third
liquid permeable material. The sample then enters the
wicking material containing the capture zone. Patent
application WO 92/01226 describes a lateral flow device
10 in which the labeled specific binding reagent is
retained in the dry state either in a zone on the
carrier material or in a separate porous body through
which the sample passes en route to the porous carrier
material of the test strip.

15 U.S. Patent Application 08/444,238 and its
corresponding PCT application 96/04748 also describe
lateral flow assay devices in which the labeled reagent
for the analyte is located in a discrete zone of the
porous carrier material of the analyte detection region.

20 Procedures using chromogenic and fluorescent dyes
as labels in biological assay procedures are also known.
Typical assay protocols call for direct or indirect
binding of a dye label to an analyte or analyte analog
in a biological sample, where the presence or absence of
25 the dye at a particular stage of the assay can be

5 determined visually and related to the amount of analyte initially present in the sample. A wide variety of specific assay protocols exist.

10 A number of those assays utilize naturally colored or dyed particles as a label, where the particles are bound to an antibody or other specific binding substance. Suggested particles include dyed latex beads, dye imbibed liposomes, erythrocytes, metal sols, and the like. The colored particle in such complexes can serve as a visible marker, where separation, capture, or aggregation of the particles is mediated through binding of the antibody or other specific binding substance. The amount of label thus segregated in a particular assay step is related to the amount of analyte initially present in the sample.

20 U.S. Patent No. 4,863,875 describes compositions comprising at least ten dye molecules or monomers covalently attached to an antibody through an isocyanate group on the dye. U.S. Patent No. 4,703,017 describes a solid phase assay device which relies on specific binding of a ligand-label conjugate on a solid support,

25

5 where the label is disclosed as a particle, such as a
liposome, or polymer microcapsule.

For example, U.S. Patent No. 4,943,522 describes
methods of detecting analytes using a solid phase
lateral flow assay where the sample is applied to a
10 lateral flow membrane having a pore size of 1-250
microns. PCT Publication WO 92/12428, which is related
to the above patent, represents an improvement on that
method and device wherein nonbibulous lateral flow is
used to conduct visible moieties, especially labeled
15 particles, e.g., dyed latex, red blood cells or
liposomes capable of reacting with analyte or a
competitor thereto into a capture zone for detection,
using a bibulous support made nonbibulous by treatment
with a blocking agent. The result is a one-step assay
20 which can be conducted in a very short period of time
(typically, within 60 seconds), and wherein the readout
is usually available instantaneously upon the sample
contacting a capture zone.

Immunoassays have been developed to detect the
25 presence or absence of a variety of analytes including

5 analytes useful in clinical diagnoses, including the
diagnosis of Group A Streptococcus.

Group A Streptococcus is one of the most important
causes of acute upper respiratory tract infection.
Approximately 19% of all upper respiratory tract
10 infections are caused by Group A Streptococci. (Lauer,
B.A., et al., J. Clin. Microb., 17:338-340 (1983)).
Early diagnosis and treatment of Group A Streptococcal
pharyngitis has been shown to reduce the severity of
symptoms and further complications such as rheumatic
15 fever and glomerulonephritis. (Youmans, G. P., et al.,
in The Biologic and Clinical Basis of Infectious
Diseases, at 177-183 (W.B. Saunders Co. 1980)).
Conventional identification procedures for Group A
Streptococcus from throat swabs involve the isolation
20 and subsequent identification of viable pathogens by
techniques that require 24 to 48 hours or longer.
(Faklam, R.R. and Washington, J.A., Streptococcus and
Related Catalase-Negative Gram-Positive Cocci, in Manual
of Clinical Microbiology at 238-257 (Balows, A. et al.,
25 eds., 5th ed. 1991)). Immunoassays which detect Group

5 A Streptococcal antigens can be performed in less than
one hour.

Various immunoassays for the detection of Group A
Streptococcus from throat swabs are commercially
available. Immunoassays for Group A Streptococcus sold
10 by Applied Biotech, Inc. and Abbott require the
transfer/application of a solubilized sample to the
immunoassay device and the addition of three reagents in
a specific order. In the Abbott test, the throat swab
sample is extracted in a mixture of 3 drops of 2.0 M
15 sodium nitrite and 3 drops of 1.0 M acetic acid which
are mixed just prior to the extraction procedure. The
swab is introduced into this solution, and twirled to
obtain mixing. The sample solution is then neutralized
with 3 drops of a solution of 1.0 M Tris buffer prior to
20 running the immunoassay. The immunoassay device
contains a plastic housing with a sample well into which
the extracted sample is poured.

Similarly, in the Applied Biotech test, sold under
the trademark "SURESTEP", the throat swab sample is
25 extracted in a mixture of 3 drops of 1.0 M sodium

5 nitrite and 3 drops 1.25 M acetic acid which are mixed
just prior to the extraction procedure. After the
extraction procedure, the sample mixture is neutralized
with 3 drops of a solution of .1 M Tris-0.7 M sodium
hydroxide prior to running the immunoassay. The
10 immunoassay device contains a plastic housing with a
sample chamber into which the extracted sample is
pipetted.

15 In addition, U.S. Patent No. 5,591,645 describes a
solid phase chromatographic immunoassay for detecting
Group A streptococcus. The assay requires the use of
three reagents which must be added in a specific order,
i.e., 0.1 M HCl was added to 4M sodium nitrite to obtain
nitrous acid. An aliquot of Group A streptococcus was
added to the nitrous acid solution. 1 M Tris base was
20 then added to the sample in order to neutralize the
nitrous acid prior to running the immunoassay on a
dilution series of samples. The end point of the test
was 5×10^5 organisms/ml.

25 Such tests requiring the addition of more than two
reagents introduce the possibility of user error in the

5 sequence of addition of the reagents, necessitating
performance of the test by a skilled worker in order to
obtain reliable results.

Other tests which do not require transfer of a
sample after extraction have complex housings with an
10 area designed for in-the-device sample extractions. The
housing of these tests contain an area designed to
receive the swab, and requires use of specially designed
swabs which fit precisely into the swab chamber area.

The Binax "NOW" Strep A test consists of a complex
15 folding booklet cardboard housing which contains an area
on the inner right side into which the swab is inserted
between layers of cardboard. 4 drops of 2 M sodium
nitrite with Tween 20 detergent and 4 drops of .125 M
acetic acid with Tween 20 are then added to the swab
20 area, and the swab is rotated. This requires use of a
specially designed swab which will fit into the hole
designed to receive the swab. The cardboard housing is
then folded, bringing the immunoassay strip, housed in
the left inner surface, into contact with the swab.

25 The Quidel "QUICKVUE" Test contains a complex

5 plastic housing having a specially designed "in-line"
swab chamber into which a specially designed swab is
inserted. The "QUICKVUE" test contains an extraction
solution bottle obtaining .6 ml of 4M sodium nitrite
with 0.01% Thimerosal, and an internal crushable ampule
10 of .65 ml .2 M acetic acid. The ampule is crushed to
mix the solutions just prior to sample extraction. The
throat swab specimen is inserted into the swab chamber,
and 8 drops of the freshly mixed solution are added to
the swab chamber. As the liquid seeps through the swab,
15 the liquid is carried by capillary action into the test
strip. Use of a swab which does not precisely fit into
the plastic chamber will result in liquid flow which is
too rapid for efficient sample extraction.

These one-step assays are complex devices requiring
20 a number of immunoassay reagents. Moreover, because the
geometry of these devices for these one-step assays
limits the amount of mixing of the sample with the
extraction reagents and/or the time of exposure of the
sample to the extraction reagents, these assays have
25 limited sensitivity due to poor extraction of the

5 analyte. Thus, there is a need for a one-step assay
which permits thorough mixing of the sample with the
extraction reagents, and exposure of the sample to the
extraction reagents for a desired length of time.
Simplifying the number of reagents added during the
10 performance of the assay and eliminating the need to
transfer the sample after extraction is also desirable
in an assay for health and safety and regulatory
purposes. When two reagents are added to the sample
during performance of the assay, simplification can also
15 be obtained if the reagents are not required to be added
to the sample in a particular sequence.

Thus, there is still the need to develop an
immunoassays for a Strep A antigen extracted or
solubilized from samples, where 2 or less sample
20 extraction reagents are added, in no particular
sequence, during performance of the assay, and where the
sample does not require transfer to the immunoassay
device after efficient sample extraction. There is also
a need for a one-step assay utilizing devices which do
25 not require complex plastic or cardboard housings or

5 specially designed swabs to obtain sample extraction.

None of the references or products described herein
is admitted to be prior art.

SUMMARY OF THE INVENTION

10 This invention relates to an immunoassay for
extracted Streptococcus Group A carbohydrate antigens
which can be performed by individuals without extensive
training in laboratory techniques. Moreover, these
assays do not require transfer of the sample to the
15 immunoassay device following extraction of the antigens.

The lateral flow immunochromatographic assay
devices of the present invention do not require the use
of elaborate cardboard or plastic casings or specially
fitted swabs. Instead commercially available test tubes
20 and swabs may be used with the assays of this invention.

The methods of this invention provide for the
detection of analytes in samples which must be extracted
prior to running the assay, while minimizing sample
manipulation. In a first aspect, this invention relates
25 to: A method to determine the presence or absence of

5 Streptococcus Group A antigen in a sample, comprising
the following steps:

(a) extracting the antigen from said sample in an
assay chamber with two or less extraction reagents,
wherein said two reagents may be added to said assay
10 chamber in no particular sequence;

(b) introducing a lateral flow
immunochromatographic assay device into said extraction
reagents containing said extracted antigen without
further addition of reagents or manipulation of said
15 sample;

(c) forming an antigen-indicator labeling reagent
complex; and

(d) determining the presence or absence of said
antigen in the sample by the presence or absence of a
20 signal formed by the binding of said antigen-indicator
labeling reagent complex to an indicator capture reagent
specific for said antigen-indicator labeling reagent
complex.

In a preferred embodiment, the method of this
25 invention further comprises the step of determining the

5 presence of a positive control signal. The positive
control signal acts as an internal control that the
reagents are functional and the assay has been performed
properly.

10 By stating that the extraction reagents may be
added to said assay chamber in no particular sequence
means that the order of addition of the extraction
reagents will not affect the assay results. One of
ordinary skill in the art would recognize that even if
one of the extraction reagents contains a color
15 indicator which would make it preferable for the colored
reagent to be added to the assay chamber first, the
results of the assay will not be affected by the order
in which the extraction reagents are added to the assay
chamber.

20 In one preferred embodiment, extraction of the
Strep A antigen preferably is carried out by mixing a
first solution of sodium nitrite, and a second solution
of acid, preferably acetic acid, to obtain nitrous acid
in a test tube, inserting a throat swab into the
25 solution, and vigorously mixing the sample with the

5 reagents by turning the throat swab against the side of
said test tube. Preferably the concentration of the
sodium nitrite solution is 0.2-5 M, while the
concentration of the acetic acid solution is preferably
10 .02-2 M. More preferably, the extraction is carried out
by contacting the throat swab with a freshly prepared
solution of nitrous acid. The freshly prepared solution
of nitrous acid is preferably made by mixing equal
volumes of 2 M sodium nitrite and 0.3 M acetic acid in a
test tube, and vigorously turning the swab against the
15 side of the test tube, preferably at least 10 times.
After vigorously mixing the sample with the reagents,
extraction is allowed to proceed for preferably at least
10 seconds, more preferably for at least 60 seconds to
allow adequate extraction of the carbohydrate antigen.

20 By freshly prepared is meant that the solution must
preferably be mixed not more than 30 minutes prior to
the extraction of the antigen, more preferably not more
than 2 minutes prior to the extraction.

The sodium nitrite solution may also contain a
25 detergent, for example, Tween 20 detergent, or an

5 antibiotic, for example, 0.01% Thimerosal.

10 In an even more preferred embodiment, a color indicator is added to the solution of 2 M sodium nitrite, so that as the 0.3 M acetic acid solution is added to the solution of 2M sodium nitrite, and the color of the 2M sodium nitrite solution changes from pink to light yellow.

15 In the assays of this invention, neutralization of the nitrous acid solution is not required following extraction of the antigens prior to running the lateral flow immunochromatographic assay. Preferably the lateral flow immunochromatographic assay device contains a porous sample receiving region member impregnated with buffer which will neutralize the nitrous acid during lateral flow of the sample through the device.

20 In particular, the invention can be used to detect the presence or absence of the Streptococcus Group A antigen in samples requiring extraction of the antigen, preferably throat swabs.

25 Thus, the first aspect of the present invention features immunochromatographic assays for the detection

5 of the presence or absence of Strep A antigen in a sample which requires extraction prior to performing the immunochromatographic assay. These one-step assays preferably require addition of two or less immunoassay reagents to the assay chamber.

10 Because simplifying the number of reagents added to the sample and decreasing manipulation of the sample following extraction is desirable for health and safety and regulatory purposes, it is desirable to develop other design variations that simplify the number of
15 reagents necessary to perform the assay, and which eliminate the need for further sample manipulation following extraction of the antigen in order to decrease the possibility of user error. For instance, further manipulation of the sample can result in mixing or loss
20 of samples during transfer. In addition, use of more than three reagents which must be added to an assay chamber in a specific order can result in errors in the sequence of addition.

Any one-step immunochromatographic assay device can
25 be used in the assays of this invention, including test

5 strip devices or devices having plastic housings.
Preferably, the immunochromatographic assay device
contains a sample receiving region which is made of a
porous material. The porous material conducts lateral
flow of the liquid sample. The sample receiving region
10 is in contact with an analyte detection region.
Lateral flow of a sample containing extracted antigens
will continue from the sample receiving region to the
analyte detection region. The sample receiving region
and the analyte detection region may be present on a
15 single porous member, or may comprise at least two
separate porous members in lateral flow contact.

Preferably the analyte detection region contains
mobile labeling reagents located at a discrete situs.
These mobile labeling reagents may include an indicator
20 labeling reagent and a control labeling reagent. The
mobile indicator labeling reagent consists of a first
reagent, preferably a monoclonal or polyclonal antibody,
that specifically binds the analyte to be detected.
Attached to the antibody, either covalently or
25 noncovalently, is a substance or particle capable of

5 producing a signal detected visually. Such particles used as labeling reagents can be colloidal gold, dye sols, colored latex and the like.

Preferably, the mobile indicator labeling reagent is a rabbit antibody to the carbohydrate antigen of Streptococcus Group A, and the label is colored latex (Blue).

The mobile control labeling reagent is a particle or molecule which does not bind to the indicator capture reagent and is conjugated to a substance or particle capable of producing a signal. For instance, the control labeling reagent may be BSA conjugated to colored latex (Red), while the control capture reagent is anti-BSA.

Alternatively, the control labeling reagent may be the same reagent as the indicator labeling reagent. In that embodiment, the "control capture reagent" is a reagent capable of binding the control labeling reagent but which does not bind to the antigen analyte. In that embodiment the indicator capture reagent binds to an epitope of the analyte, which is also bound by the

5 mobile indicator labeling reagent. It is well known in
the art that the carbohydrate antigen of Group A
Streptococcus contains a repeated epitope. Thus, a
sandwich complex can be formed even if the indicator
capture reagent and the indicator labeling reagent each
10 contain an antibody to the same epitope of Strep A.

One skilled in the art will recognize other
suitable labeling particles, including gold sol
particles, or other colored latex particles. One of
ordinary skill in the art will also appreciate that the
15 label can be the same on the indicator reagent and the
control reagent. The indicator labeling reagent and
control labeling reagent may be the same or different
reagents.

The analyte detection region also preferably
20 contains an immobile indicator capture reagent at a
discrete situs. In addition, the analyte detection
region also preferably contains an immobile control
capture reagent at a discrete control situs.

The analyte detection region is also in lateral
25 flow contact with an end flow region. The end flow

5 region contains a porous material which conducts lateral
flow of the liquid sample. It is capable of absorbing
excess liquid sample. The end flow region may be on the
same porous member as the analyte detection region, or
may be a separate porous member in lateral flow contact
10 with the analyte detection region.

In addition, in an embodiment using a test strip,
the porous materials in the above aspect are laminated
with one continuous or separate semi-rigid material,
preferably at least 0.001 inches thick. The total
15 thickness of all of the layers of the immunoassay device
is preferably at least 0.003 inches thick. The laminate
covers the back only and provides adequate mechanical
strength to the device, i.e., it provides support and
strength characteristics to the porous material and
20 overall device such that lateral flow of liquid through
the device will not be interrupted, for instance by the
collapse or disintegration of the device upon wetting.
Additional support for the device during the immunoassay
may be provided by the walls of a test tube against
25 which the device may rest during the lateral flow.

5 The term "assay chamber" refers to any liquid-proof container to which reagents can be added and into which the lateral flow immunochromatographic assay device can be inserted after sample extraction. Preferably the assay chamber is a test tube.

10 In an alternate embodiment, the assay chamber may be branched, e.g., U-shaped or V-shaped (Fig. 6), where the two branches are joined at the bottom and are in communication with the sample reservoir. The swab may be inserted into one branch of the assay chamber, while
15 the device may be inserted into a second branch.

 In still another embodiment, the assay chamber may be made of non-rigid plastic, and contain a crushable ampule containing one of the reagents. Extraction of the sample may be initiated by squeezing the tube to
20 crush the ampule to obtain mixing of the reagents.

 Alternatively, one reagent may be placed in a crushable ampule which can be inserted into the bottom of an assay chamber made of a rigid material such as plastic, glass, or metal, and the ampule may be crushed
25 with the swab.

5 The term "manipulation" refers to transfer of the
sample from one container to a second container or
surface, and includes for example, pouring or pipetting
the extracted sample from a test tube into or onto an
immunoassay device. For example, manipulation of the
10 sample following extraction may be the pouring or
pipetting of the processed sample from a test tube into
the plastic housing of an immunoassay device, where the
processed sample contacts the lateral flow test strip of
the device.

15 "Processing" a sample refers to exposing a solid
sample or a non-homogeneous liquid sample to a reagent
in order to extract, or make accessible, an analyte to
the indicator labeling reagent during the lateral flow
assay. By solid or non-homogeneous liquid sample is
20 meant a sample which comprises a solid phase or a liquid
sample which is adsorbed to a solid phase. Preferably,
the solid or non-homogeneous liquid sample may be a
swab. Preferably the swab is a throat swab.

25 To "extract" the analyte during processing means to
make the binding site to which the labeling reagent will

5 bind accessible to the binding agent during the lateral
flow assay. This extraction may be, for instance,
cleavage of the carbohydrate antigen from the cell wall
of Group A Streptococcus, or disruption of cell walls or
membranes to expose membrane bound analytes or
10 intracellular analytes. Preferably the processing will
extract a sufficient percent of the analyte present in
the sample such that 4×10^5 cells of Group A
Streptococcus /swab can be detected.

15 The term "analyte" as used herein refers to a
compound or composition to be detected or measured in
the test sample. The analyte will have at least one
epitope that an antibody or an immunological reactive
fragment thereof can recognize. Analyte can include any
antigenic substances, haptens, antibodies and
20 combinations thereof. The analyte of interest in an
assay can be, for example, a protein, a peptide, an
amino acid, a nucleic acid, a hormone, a steroid, a
vitamin, a pathogenic microorganism for which polyclonal
and/or monoclonal antibodies can be produced, a natural
25 or synthetic chemical substance, a contaminant, a drug

5 including those administered for therapeutic purposes as
well as those administered for illicit purposes, and
metabolites of or antibodies to any of the above
substances. One preferred example of an analyte
suitable for detection is the Group A Streptococcal
10 antigen from throat swabs.

Preferably the extraction of sample is carried out
in a 10 X 50 mm test tube (Evergreen) using a 100% rayon
swab (PurFybr). Following the sample extraction, the
immunoassay is initiated by inserting the device into
15 the processed sample. The swab may be removed from the
test tube or moved to the side of the test tube upon
insertion of the device.

In still another embodiment, introduction of the
lateral flow device into the processed sample without
20 further sample manipulation may be indirect. For
instance, the test tube may have a sealable lid which is
liquid-proof when sealed, and the inner surface of the
top may have the lateral flow assay device mounted at a
perpendicular angle to the inner surface of the lid.

25 When sealed, the lateral flow assay device will extend

5 into the test tube, parallel to the sides of the test
tube. After processing of the sample, the top of the
test tube can be inserted into the test tube and sealed.
After this introduction of the lateral flow device into
the test tube, the test tube can be inverted to bring
10 the processed sample containing the solubilized analyte
into contact with the lateral flow assay device.

The term "sample" as used herein refers to any
biological sample that could contain an analyte for
detection which requires extraction prior to performing
15 the immunoassay. Preferably, the sample is a throat
swab sample.

As used herein, the term "sample receiving region"
means the portion of the assay device which is in direct
contact with the liquid sample, *i.e.*, it receives the
20 sample to be tested for the analyte in question. The
liquid sample can then migrate, through lateral flow,
from the sample receiving region towards the end flow
region. Preferably the sample receiving region is the
edge of the assay device. The sample receiving region
25 in lateral flow contact with the analyte detection

5 region. This could either be an overlap or end-to-end
connection. The analyte in the sample must be capable
of migrating, through lateral flow, with the liquid
sample. The sample receiving region is made of porous
material, usually porous paper. Preferably the sample
10 receiving region is impregnated with buffer to
neutralize the extraction reagents during the lateral
flow immunoassay.

As used herein, the term "porous material" refers
to any material capable of providing lateral flow. This
15 would include material such as nitrocellulose,
nitrocellulose blends with polyester or cellulose,
untreated paper, porous paper, rayon, glass fiber,
acrylonitrile copolymer or nylon. One skilled in the
art will be aware of other porous materials that allow
20 lateral flow. The term "lateral flow" refers to liquid
flow in which all of the dissolved or dispersed
components of the liquid are carried at substantially
equal rates and with relatively unimpaired flow
laterally through the material, as opposed to
25 preferential retention of one or more components as

5 would occur, e.g., in materials capable of adsorbing or imbibing one or more components.

10 The term "mobile" as referred to herein means diffusively or non-diffusively attached, or impregnated. The reagents which are mobile are capable of dispersing with the liquid sample and carried by the liquid sample in the lateral flow. The term "immobile" as used herein refers to reagents which are attached to the support such that lateral flow of the liquid sample does not affect the placement of the immobile particle in the discrete region of the porous material. Such attachment can be through covalent, ionic, or hydrophobic means. Those skilled in the art will be aware of means of attachment to immobilize various particles.

20 The term "labeling reagent" as used herein refers to any particle, protein or molecule which recognizes or binds to the analyte in question or a particle, molecule, protein which does not recognize, or bind to the analyte and has attached, conjugated or bound to it, either chemically, covalently or noncovalently, or 25 ionicly or nonionicly any substance capable of producing

5 a signal that is detectable by visual or instrumental means. Such labels producing a signal would include chromogens, catalysts, fluorescent compounds, colloidal metallic and nonmetallic particles, dye particles, enzymes or substrates, organic polymers, latex
10 particles, liposomes with signal producing substances and the like. The particle or molecule recognizing the analyte can be either natural or non-natural, preferable monoclonal or polyclonal antibody.

15 Preferably the indicator labeling reagent is a label bound to a rabbit antibody to the Streptococcus Group A carbohydrate antigen unique to Streptococcus Group A. Preferably the label is colored latex particles or gold sol, for example, blue latex particles. In this preferred embodiment, a second
20 control labeling reagent is BSA bound to a label, preferably colored latex particles, or gold sol, for instance, red latex particles. If desired, the label bound to the rabbit-anti Streptococcus Group A antibodies and the BSA may be the same, for instance,
25 colored latex particles or gold sol.

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20 control labeling reagent is BSA bound to a label, preferably colored latex particles, or gold sol, for instance, red latex particles. If desired, the label bound to the rabbit-anti Streptococcus Group A antibodies and the BSA may be the same, for instance,
25 colored latex particles or gold sol.

5 The term "indicator capture reagent" as used herein
refers to any particle or molecule which recognizes or
binds the analyte in question. The indicator capture
reagent is capable of forming a binding complex with the
labeling reagent that has bound to the analyte in the
10 sample. The indicator capture reagent is immobilized to
the porous material of the analyte detection region.
The indicator capture reagent is not affected by the
lateral flow of the liquid sample due to the
immobilization to the porous material. The particle of
15 molecule can be natural, or non-natural, i.e., synthet-
ic. Once the capture reagent binds the analyte-
indicator labeling reagent complex it prevents the
analyte-indicator labeling reagent complex from
continuing with the lateral flow of the liquid sample.

20 The term "control capture reagent" as used herein
refers to any particle or molecule which is capable of
binding a control labeling reagent. For example, the
control labeling reagent may be BSA conjugated to a
label, for example, to colored latex or gold sol. The
25 colored latex may be, for instance, a red latex. The

5 control capture reagent in that embodiment would be a particle or molecule which recognizes or binds the BSA conjugated to the label. In this embodiment preferably, the control capture reagent would be a monoclonal or polyclonal antibody which recognizes BSA.

10 Alternatively, the "control labeling reagent" may be the same as the "indicator labeling reagent". For instance, the control labeling reagent and indicator labeling reagent may be a rabbit anti-Strep A antibody linked to a label such as gold sol particles. In that
15 embodiment, the capture reagent for the "control labeling reagent" also binds to the "indicator labeling reagent", but it does not bind the analyte. For instance, the capture reagent for the positive control signal may be anti-rabbit γ -globulin antibody, while the
20 capture reagent for the analyte signal is an antibody to the Strep A antigen.

The control reagent is immobilized to the porous material. Once it binds the control reagent it immobilizes the labeling reagent and prevents it from
25 continuing lateral flow with the liquid sample, giving

5 rise to a positive control signal. Just as the capture
reagent is immobilized in a discrete situs on the porous
material of the analyte detection region, the control
reagent is also immobilized in a discrete situs on the
porous material of the analyte detection region. Binding
10 of the immobilized capture control reagent to the
control labeling reagent results in the formation of a
positive control signal, which serves as an internal
control that the assay was performed properly. The
capture reagent for the control reagent may be applied
15 to the porous in any geometrical shape desired.

The term "analyte detection region" as used herein
refers to the portion of the assay device which is in
lateral flow contact with the porous material of the
sample receiving region and the end flow region. The
20 contact can be an overlap or end-to-end connection, or
the analyte detection region may be found on the same
porous member as the sample receiving region and the end
flow region.

The analyte in the sample must be capable of
25 migrating through lateral flow with the liquid sample.

5 The analyte detection region is made of a porous material just as the sample receiving region is. Preferably, the analyte detection region is made of nitrocellulose. The sample receiving region, the analyte detection region and the end flow region can be made of different material, or can be separate regions of the same porous member. The analyte detection region can contain the mobile labeling reagents, the immobile indicator capture reagent and the immobile control capture reagent. In other embodiments the analyte detection region contains only the immobilized control capture reagent and the indicator capture reagent.

The term "discrete capture situs" or "discrete control situs" as used herein refers to a defined area in which either the labeling reagents, the capture reagent or the control reagent are impregnated (for the labeling reagents) or immobilized (for the capture reagents) to the porous material. The discrete capture situs of the control or the capture reagents provide a discrete visible signal in a desired geometric shape from which to view the results of the test. For

5 example, if the one labeling reagent is anti-analyte
conjugated to label such as colored latex or gold sol,
then a discrete signal will appear at the discrete
capture situs if the indicator capture reagent binds and
immobilizes the indicator labeling reagent complex. If
10 the control labeling reagent is BSA conjugated to a
label, such as colored latex or gold sol, then a
discrete signal will form at the discrete control situs
if the capture control reagent immobilizes the BSA-
control labeling reagent.

15 The term "end flow region" as used herein refers to
the portion of the assay device which is in lateral flow
contact with the analyte detection region. The liquid
sample migrates into the sample receiving end of the
device, through the device to the opposite end flow
20 region. The end flow region is capable of absorbing
excess liquid sample. The contact with the analyte
detection region can be either by overlap or end-to-end
connection. Alternatively, the end flow region may be a
region on the same porous member as the analyte
25 detection region. The end flow region is made of porous

5 material, usually porous paper.

10 The term "semi-rigid" as used herein refers to the material used to support the porous material of the device. This can be one continuous piece of laminate or separate pieces. The laminate is preferably vinyl but one skilled in the art will recognize that numerous materials can be used to provide the semi-rigid support. The semi-rigid material is preferably at least 0.001 inches thick. Preferably the total thickness of the immunoassay device will be 0.003 inches thick. The total thickness of the immunoassay device consists of the thickness of the backing, the membrane elements, label pads (if desired), and the cover. This minimum total thickness is required in order to produce the desired adequate mechanical strength or support for the device to function effectively.

15 The term "adequate mechanical strength" as used herein refers to a desired support to the assay device so as to function properly. The adequate mechanical strength is the support achieved for the entire assembled assay device so as to function properly in the

20

25

5 collection and analysis of the analyte in the liquid
sample. The minimum adequate mechanical strength is a
total thickness of the device of 0.003 inches thick.
This preferred measurements will provide sufficient
strength and support to the porous material and assay
10 device such that no interference with the lateral flow
results, for instance from the collapse or
disintegration of the device upon wetting.

The term "plastic material," or "plastic cover," or
"cover" as used herein refers to any plastic material
15 which can cover the porous material of the device.
Preferably, this is mylar, however, those skilled in the
art will know of various materials that can be used for
such purposes. The cover can be one continuous plastic
or separate pieces as shown in the figures. It must
20 allow the discrete control and discrete capture situses
to be viewed. Thus, if the cover is clear then the
result can be viewed through the clear cover. If the
cover is not clear, then a window, gap or hole must be
used so the results can be viewed. In addition, the
25 cover must leave a portion of the sample receiving

5 region exposed so the sample can be applied to the receiving region.

Alternatively, the backing and plastic cover can be a molded plastic housing.

10 Other features and advantages of the invention will be apparent from the following detailed description of the invention in conjunction with the accompanying drawings and from the claims.

Description of the Drawings

15 Figure 1 illustrates an expanded perspective view of the immunochromatographic elements assembled into a test device according to a preferred embodiment of the present invention.

20 Figure 2 illustrates an expanded perspective view of the immunochromatographic elements assembled into a test device according to an alternative preferred embodiment of the present invention.

25 Figure 3 illustrates an expanded perspective view of the immunochromatographic elements assembled into a test device according to another alternative preferred

embodiment of the present invention.

Figure 4 illustrates an upper view of the test device constructed according to the present invention having upper covering printed with product information.

Figure 5 illustrates the mixing of reagents in a test tube.

Figure 6 illustrates placement of a throat swab into the test tube containing the reagents.

Figure 7 illustrates the placement of the device into the test tube containing the solubilized sample.

Figures 8(a)-(c) illustrate the interpretation of results.

Figure 8(a) shows a positive result. A test signal line is formed by binding of the indicator capture reagent to the indicator labeling reagent-Strep A complex. A positive control line is formed by binding of the control capture reagent to the control labeling reagent.

Figure 8(b) shows a negative result. Only a positive control line is formed by binding of the control capture reagent to the control labeling reagent.

5 Figure 8(c) shows an invalid result. If no
positive control line has appeared or the background is
too high and it is not possible to see the positive
control signal, the result is invalid.

10 Figures 9(a)-(b) illustrate embodiments of
branched assay chambers.

15 Figure 9(a) shows a V-shaped assay chamber, while
Figure 9(b) shows a U-shaped assay chamber which can be
used in one embodiment of this invention. In these
embodiments, the swab can be inserted into one branch of
the assay chamber, while the test strip is inserted into
the second branch of the assay chamber.

 Figure 9(c) illustrates an assay chamber containing
a crushable divided ampule containing two reagents which
are not mixed until the ampule is crushed.

20 The drawings are not necessarily to scale, and
certain features of the invention may be exaggerated in
scale and shown in schematic form in the interest of
clarity and conciseness.

25 Detailed Description of the Invention

5 The following are examples of the
immunochromatographic assays of the present invention.
These examples are offered by way of illustration and
are not intended to limit the invention in any manner.
Examples of devices are also found in U.S. Serial No.
10 08/444,238, and U.S. Serial No. 08/752,695, which are
incorporated by reference.

 Figure 1 depicts a preferred embodiment of the
device used in the methods of this invention. This
device does not have a plastic or cardboard casing. A
15 series of porous material pieces (2), (3) and (4), and
(6) are laminated to an elongated strip of a semi-rigid
material (1), such as vinyl and the like.

 The separate sample receiving region (4) is a
porous material, usually paper. In this preferred
20 embodiment shown in Fig. 1, the separate sample
receiving region (4) is in direct liquid flow contact
with the separate labeling reagent region (3). The
separate labeling reagent region contains additional
indicator labeling reagent, for instance, an antibody to
25 the analyte bound to a label. The separate labeling

5 reagent region also contains additional control labeling reagent. The separate labeling reagent region is preferably made of a mixture of cellulose and polyester, or other porous material.

10 The contact between the separate sample receiving region and the separate labeling reagent region may be perpendicular flow contact, with the separate sample receiving region placed on top of the separate labeling reagent region (not shown). The separate labeling reagent region is in direct lateral flow contact with
15 the analyte detection region (2). The analyte detection region contains a discrete zone containing mobile indicator labeling reagent and control labeling reagent (2a). The mobile indicator labeling reagent in the analyte detection region is the same indicator labeling reagent found in the separate labeling reagent region
20 (3), which is capable of binding to the analyte. A strip of plastic material (5), preferably clear mylar, is covered on top of the device. Portion (5a) can be a window or clear so as to permit viewing of the capture
25 and control discrete sites, i.e., to permit viewing of

5 the results. An end zone region (6) is in lateral flow contact with the analyte detection region.

In the embodiment shown in Fig. 1, the analyte detection region (2) of the immunochromatographic assay device contains an immobile indicator capture reagent in a discrete situs (2b) and an immobile control capture reagent at a discrete situs (2c).

10 In the embodiment shown in Figure 2, the mobile indicator labeling reagent and control labeling reagent are found in the separate labeling reagent region, and no additional labeling reagents are placed on the membrane of the analyte detection region. In the embodiment shown in Figure 3, there is no separate labeling reagent region. All of the mobile indicator labeling reagent and control labeling reagent are placed in the analyte detection region (2A).

20 The mobile labeling reagents consist of a first reagent, preferably a monoclonal or polyclonal antibody, that specifically binds the analyte to be detected. This reagent is also called the indicator labeling reagent. Attached to the antibody, either covalently or

25

5 noncovalently, is a substance or particle capable of
producing a signal detected visually. Such labeled
particles used can be colloidal gold, dye sols, colored
latex and the like. Preferably, the label is latex
(Blue). One skilled in the art will recognize suitable
10 labeling particles.

The second mobile labeling reagent is a particle or
molecule which does not recognize the analyte and is
conjugated to a substance or particle capable of
producing a signal. This second reagent is referred to
15 as the control labeling reagent. Preferably, the
control labeling reagent is BSA conjugated to latex
(Red).

In an assay, the sample receiving region (4) of the
assay device is directly placed into a sample containing
20 extracted analytes, for example, a processed throat swab
sample containing extracted Streptococcus Group A
carbohydrate antigen. Preferably the antigens are
extracted using two or less reagents. The sample flows
laterally along the porous material region by capillary
25 action and migrates past the separate labeling reagent

5 region (3), and then past the labeling reagents in the
analyte detection region (2a). The presence and/or the
amount of analyte in the sample may then be determined
by the visibility of a signal line (2b) formed by the
specific binding of the immobilized indicator capture
10 reagent to the analyte-indicator labeling reagent
conjugate complex.

The appearance of a second signal (2c) may be
utilized as a built-in positive control signal. This
positive control signal results from binding of the
15 immobilized control capture reagent to the control
labeling reagent, e.g., BSA-Red latex. If the reagents
and assay are working properly, then a red signal line
will appear at (2c) the discrete control situs. The red
control line is an internal control. The test stick
20 must absorb the proper amount of the sample and the test
stick must be working properly for the red control line
to appear. For the test stick to be working properly,
the capillary flow must occur. Thus, the control line
serves as an indication that the proper amount of
25 reagents have been added to the assay chamber, and that

5 sufficient lateral flow has occurred for the control
labeling reagent to reach the control capture reagent
zone.

 The results of an assay can then be observed
through a viewing window (5a) covered by clear mylar.

10 The device is required to have an adequate total
mechanical strength (as defined above and discussed
below) in order for the device to function without
disruption of lateral flow.

15 Other layouts, for instance, of the upper covers or
the labeled particles are possible, as long as lateral
flow of the porous membranes is permitted. Overlap or
end-to-end connection can be used as long as lateral
flow occurs. Alternatively, the various regions of the
test strip may also be placed on a single porous member.

20 For example, the control labeling reagent and
indicator labeling reagent may be placed only in a
region of the analyte detection region, and the separate
labeling reagent region may be omitted. Alternatively,
the control labeling reagent and the indicator labeling
25 reagent may be placed only in a separate labeling

5 reagent region, and additional indicator labeling
reagent or control labeling reagent may be omitted from
the analyte detection zone.

10 The assays as described above in the Summary of the
Invention provide a method for antigen extraction from
the sample and introduction of the device into the
sample containing extracted analytes without the need
for specimen manipulation following the extraction.
This provides an advantage of a more rapid and
convenient test procedure to the user.

15 Dimensions of the Exemplary Assay Device

Upper Covering:	4 mm x 98 mm
Lower Backing:	4 mm x 98 mm
Separate Labeling Reagent Region:	4 mm x 5 mm
Sample Receiving Region:	4 mm x 20 mm
End Flow Region:	4 mm x 56 mm
Analyte Detection Region:	4 mm x 25 mm
Viewing Window:	4 mm x 9 mm

5 (Note: Product information may be printed on the
upper covering as shown in Figure 4.)

In yet another aspect, the present invention
comprising an immunochromatographic assay device without
molded plastic casings greatly reduces the cost for
manufacturing. In addition, the advantage of using a
10 same basic design with universal applicability for
different analytes also promotes the objective of
inventory reduction.

15 **EXAMPLE 1--One-Step Immunoassay for Strep A Which Does
Not Require Sample Manipulation**

Most preferably the one-step assay device will
contain an OSOM™ Strep A Test . The OSOM™ Strep A Test
20 detects either viable or nonviable Group A Streptococcus
organisms directly from a throat swab, providing results
within 5 minutes.

Specimens may be collected with a sterile swab from
the tonsils and/or the back of the throat, taking care
25 to avoid the teeth, gums, tongue or cheek surfaces.
Sterile swabs may be used to collect the specimens.

5 Preferably sterile rayon or dacron swab are used to
collect specimens. Alternately, swabs with transport
tubes containing liquid media can also be used.
Preferably the liquid media used in transport tubes will
be Modified Stuart's Transport Media ("CULTURETTE"
10 available from Becton Dickinson).

The OSOM™ Strep A Test can be used for the
qualitative detection of Group A Streptococcal antigen
from throat swabs or confirmation of presumptive Group A
Streptococcal colonies recovered from culture.

15 **Materials used and preparation of Strep A Test:**

1. Analyte Detection Region: Important features of
the material are its ability to wick fluids and to bind
20 proteins. Exemplary materials include nitrocellulose,
nylon or the like. In a preferred embodiment of this
invention, the material is nitrocellulose with or
without laminated solid support such as polyester.
Nitrocellulose is readily available from numerous
25 suppliers.

5 2. Sample Receiving Region: Suitable materials
include cotton, cellulose, mixed fibers, glass fiber and
the like. For example, paper such as 470 and 740-E from
Schleicher and Schuell, Keen, NH, or D28 from Whatman,
Fairfield, NJ, can be selected for its high fluid
10 absorption and wicking speed. A more porous material
such as glass fiber #66078 from Gelman Sciences, Ann
Arbor, MI, or "POREX" from Porex Technologies, Fairburn,
GA, is suitable for impregnating labeled particles.

15 3. Separate Labeling Reagent Region: A good candidate
would be a porous material which allows the ease of
releasing the impregnated labeling reagents from the
region. Such materials include glass fiber from Gelman
Sciences, Ann Arbor, MI, or Accuwik from Pall
BioSupport, Port Washington, NY.

20 4. Backing Supports: For the present invention, the
preferred materials are clear mylar with thickness about
0.001 inches to 0.010 inches for the upper covering and
white vinyl with thickness about 0.001 inches to 0.030
inches for the lower backing. Both the mylar and the
25 vinyl sheets have adhesive on one side so as to attach

5 the porous material. Materials such as mylar,
polyester, and vinyl with adhesive are readily
available.

10 5. Labeling Reagents: A chromogenic particulate such
as colored latex, colloidal gold, selenium or the like
is labeled with a suitable reagent specific for the
targeted analyte. For the present invention, the
preferred chromogenic particulate is colored latex.
More preferably, blue or red colored latex is used.
Latex is commercially available from a number of
15 sources.

20 6. End Zone Region: Suitable materials include cotton,
cellulose, mixed fibers, glass fiber and other like
materials with high fluid absorption capacity. For
example, paper such as 470 and 740-E from Schleicher and
Schuell, Keen, NH, or D28 from Whatman, Fairfield, NJ,
can be selected for its high fluid absorption and
wicking speed.

25 7. Strep A antibody: New Zealand white rabbits were
injected with partially purified Group A Streptococcus
antigen. The rabbits which produced a high titer of

antibody were identified by an enzyme immunoassay method. The sera from these rabbits were pooled and purified through Strep A antigen affinity column.

8. Anti-BSA Antibody: Affinity purified sheep anti-BSA antibody was obtained from Bethyl Lab, Montgomery TX.

9. Preparation of Latex Conjugates

The basic protocol for conjugation of protein to latex, by simple adsorption or by covalent binding, is well known in the art and is hereby incorporated by reference.

For example, the indicator labeling reagent may be an anti-Group A streptococcus antibody conjugated with blue latex, while the indicator capture reagent may be an anti-Group A streptococcus captive antibody.

Blue carboxylated latex particles (0.2 to 0.5 microns) were activated with 0.2% EDAC in the presence of 0.1% sulfo-NHS in 20 mM MES buffer, pH 5.5, for 30 minutes at room temperature. The excess amount of reagents were removed by washing in an Amicon Concentrator. The activated latex particles were

5 resuspended in 2 mM MES buffer, pH 6.5 to a
concentration of 0.5%, and a ratio of 0.05 mg Strep A
antibody were added to 1 mg of latex. The mixture was
incubated at room temperature for 2 hours. After
incubation, the conjugated latex was washed again to
10 remove free antibody. The antibody-latex conjugate was
then sonicated, filtered, and resuspended in buffer
containing 20 mM Tris, pH 8.5; 20% sucrose; 0.5% casein.

The conjugation of BSA to red carboxylated latex
(size of 0.2 to 0.5 microns) was essentially the same as
15 described above except replaced the blue latex with red
latex and Strep A antibody with BSA.

10. Preparation of Latex Coating Solution

The blue latex solution and the red latex solution
were mixed at a ratio from 5:1 to 1:1 depends upon the
20 sensitivity of the conjugate and intensity of red
control line desired. The preferable ratio is
approximate 1:1. These solutions are then impregnated
into the porous material using methods well known in the
art, all of which are hereby incorporated by reference.

5 11. Coating of Capture Reagents on the Discrete Situses
 of the Porous Material

 Thin lines of the indicator capture reagent or
control capture reagent were applied on the material
using airbrush techniques (Iwata, model HP-BC2). The
10 width of the lines can be 0.2 mm to 2 mm, a width of 1
mm is preferred. Such material is immobilized by
techniques well known in the art, hereby incorporated by
reference.

15 12. Coating of Latex Conjugate (Labeling Reagents) on
 the Material

 Immediately after the capture reagents were applied
on the material. The latex solution can be applied on
20 the material by using airjet techniques such as BioDot
Biodoser machine from Bio-Dot, Inc., Irvine, CA. The
membrane strip is then dried in a force air oven at 70°C
for 45 minutes. Such application allows the labeling
reagents to be mobile.

25 13. Preparation of Separate Labeling Reagents Region

 The separate labeling reagents region is prepared by
saturating a piece of porous material such as Accuwik
with the prepared latex coating solution. The soaked

5 material is then dried in a force air oven at 70°C for
30 minutes.

14. Preparation of Sample Receiving Region

10 In this invention, the sample receiving region not
only absorbs and transports liquid sample, it also func-
tions as a specimen collection apparatus and as a
neutralizing agent for the acidic extraction solution.
The sample receiving region may comprise a paper treated
with buffer, detergents, blocking proteins and the like
to facilitate movement of dried latex particles or to
15 reduce nonspecific binding of the assay. In the case of
the Strep A assay, 740E paper was soaked in a buffer
solution, dried, and then assembled into the assay
device. Specifically, buffer solution containing 1.5%
zwittergent 3-12, 0.1% rabbit gamma globulin, 0.1 M NaCl
20 and 0.2 M Tris, pH 9.0 was used.

15. Assembly of the Assay Device

A sheet of white vinyl (98 mm x 200 mm) is placed
on a flat surface. The cover paper on the white vinyl
sheet is removed to expose the adhesive. A strip of the
25 analyte detection region (25 mm x 200 mm) containing

5 latex and antibody lines is attached to the white vinyl sheet. A strip of the sample receiving region (20 mm x 200 mm) is attached to the left edge of the white vinyl sheet. A separate indicator reagent region (5 mm X 200 mm) is layered between the sample receiving region and
10 the white vinyl sheet. The internal ends of the separate indicator reagent region and the sample receiving region are lying flush, and overlapping the analyte detection region by 1.5 mm. The end flow region (56 mm x 200 mm) is attached to the right edge of the
15 white vinyl sheet while overlapping about 1.5 mm on top of the analyte detection region. The cover paper from the clear mylar sheet is removed (98 mm x 200 mm) to expose the adhesive. Centering the window region of the clear mylar sheet over the capture reagent lines in the
20 analyte detection region, the clear mylar sheet is attached with the adhesive side down on top of the end flow region, analyte detection region and sample receiving region. The whole sheet is pressed with a roller to ensure the lamination is secure. The
25 laminated sheet is then cut to 4 mm wide sticks.

5 Test Procedure for running the OSOM Strep A Test:

 Just before testing, 3 drops Reagent 1 (2M sodium
nitrite) (pink) and 3 drops Reagent 2 (0.3 M acetic
acid) were added to the Test Tube (the solution should
turn light yellow). The swab (PurFybr Inc., Munster,
10 IN) was immediately inserted into the tube. Vigorously
mixing of the solution by rotating the swab forcefully
against the side of the Tube at least ten times. (Best
results were obtained when the specimen was vigorously
extracted in the solution.) The samples were left
15 standing for one minute. As much liquid as possible was
expressed from the swab by pressing the swab firmly
against the side of the Tube. The swab was discarded.
An OSOM™ Strep A Test Stick was then placed into the
extracted sample. The results were read at 5 minutes.

20

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5 Comparison of the Sensitivity of Results of the OSOM™
 Assay for Streptococcus Group A and Other One-Step
 Assays

Procedure:

10 Strep A cells were picked up from a pure culture plate
 and suspended in saline solution. Subsequent serial
 dilutions were made with saline to yield different
 concentrations of cell suspension. The cell
 concentration was determined by the optical density
15 method. OD₆₅₀ of 1 is equivalent to approximately 2x10⁹
 cells/mL in suspension. 25μL of the suspension was
 pipetted onto the tip of each of the swabs supplied by
 the manufacturers. Tests were performed within 5
 minutes after the swabs were spiked with cell
20 suspension. Tests were performed by following procedure
 described in each prospective manufacturer's directional
 insert.

Results:

Cell Qty/Swab	4×10^7	4×10^6	8×10^5	4×10^5
Wyntek OSOM™	Positive	Positive	Weak Positive	Weak Positive
Quidel	Positive	Positive	Weak Positive	Negative
Binax	Positive	Positive	Negative	Negative

These results indicate Wyntek OSOM™ Strep A Test can detect Group A Streptococcus cells when present at a concentration as low as 4×10^5 cells per swab, while Quidel's and Binax's tests can only detect Strep A cells when present at a concentration of 8×10^5 cells per swab or 4×10^6 cells per swab, respectively.

Performance of OSOM™ Strep A Test in Clinical Trials

In a multi-center evaluation, a total of 639 throat swabs were collected from patients presenting with pharyngitis. Each swab was inoculated to a sheep blood agar plate, then tested by the OSOM Strep A Test. Plates were incubated for 18-24 hours at 35° - 37° C at 5-10% CO_2 with a Bacitracin disk. Presumptive GAS colonies were confirmed with commercially available Strep A

5 testing kits.

Of the 639 total specimens, 464 were found to be negative by culture and 454 were also negative by the OSOM Strep A Test, for a specificity of 97.8%. Of the 175 specimens found to be positive by culture, 168 were also positive by the OSOM Strep A Test, for a sensitivity of 96.0%. The 95% confidence intervals were calculated to be 96.6-99.0% for specificity and 94.4-97.6% for sensitivity. Overall agreement between culture and the OSOM Strep A Test was 97.3% (622/639).

The results are summarized below:

Culture Classification	OSOM/Culture	% Correct
Negative (Specificity)	454/464	97.8%
1+ (≤10 colonies)	3/6	50.0%
2+ (11-50 colonies)	9/13	69.2%
3+ (>50 colonies)	44/44	100%
4+ (predominant growth)	112/112	100%
Total Positive (Sensitivity)	168/175	96.0%
Total (Overall Agreement)	622/639	97.3%

In addition, the OSOM Strep A Test was used to confirm the identification of Group A Streptococcus on blood agar plates. As a culture confirmation test, the OSOM Strep A Test was 100% sensitive (62/62) and 100% specific (39/39).

5 The following organisms tested at levels of
approximately 1×10^8 organisms/test were all found to be
negative when tested with the OSOM Strep A Test:

Streptococcus Group B	Enterococcus faecalis	Pseudomonas aeruginosa
Streptococcus Group C	Staphylococcus aureus	Bordetella pertussis
Streptococcus Group G	Staphylococcus epidermidis	Neisseria meningitides
Streptococcus Group G	Corynebacterium diphtheria	Neisseria gonorrhoeae
Streptococcus pneumoniae	Serratia marcescens	Neisseria sicca
Streptococcus sanguis	Candida albicans	Neisseria subflava
Streptococcus mutans	Klebsiella pneumoniae	Branhamella catarrhalis
		Hemophilus influenza

20 One skilled in the art will readily appreciate that
the present invention is well adapted to carry out the
objects and obtain the ends and advantages mentioned as
well as those inherent therein. The immunological
methods and devices for detecting analytes in biological
25 samples as described herein are presently representative
of preferred embodiments, are exemplary and not intended
as limitations on the scope of the invention. Changes
therein and other uses will occur to those skilled in
the art which are encompassed within the spirit of the

5 invention or defined by this scope with the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

10 All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual
15 publication was specifically and individually indicated to be incorporated by reference.

5

Claims

We claim:

1. A method to determine the presence or absence of Streptococcus Group A antigen in a sample, comprising the following steps:

10

(a) extracting the antigen from said sample in an assay chamber with two or less extraction reagents, wherein said two reagents may be added to said assay chamber in no particular sequence;

15

(b) introducing a lateral flow immunochromatographic assay device into said extraction reagents containing said extracted antigen without further addition of reagents or manipulation of said sample;

20

(c) forming an antigen-indicator labeling reagent complex; and

25

(d) determining the presence or absence of said antigen in the sample by the presence or absence of a signal formed by the binding of said antigen-indicator labeling reagent complex to an indicator capture reagent specific for said antigen-indicator labeling reagent

5 complex.

2. The method of claim 1 further comprising the
step of:

(a) determining the presence of a positive control
10 signal.

3. The method of claim 1 wherein said sample is a
throat swab and said extracting further comprises
vigorously mixing said throat swab in said extraction
15 reagents for at least 10 seconds.

4. The method of claim 1 wherein said extraction
reagents further comprise 0.2-5 M sodium nitrite and
0.02-2 M acetic acid.

5. The method of claim 4 wherein the sodium
nitrite solution has a concentration of 2M and the
acetic acid solution has a concentration of 0.3 M,
wherein the 0.3 M acetic acid solution is added to the
20 solution of 2M sodium nitrite, and wherein the color of
25

5 the liquid changes from pink to light yellow as said
0.3 M acetic acid solution is added to said 2M sodium
nitrite solution.

6. The method of claim 1 wherein said lateral flow
10 immunochromatographic assay device having a sample
receiving region containing neutralizing buffer.

7. The method of claim 1 wherein said lateral flow
immunochromatographic assay device further comprises a
15 test strip without a plastic housing.

8. The method of claim 1 wherein said lateral
flow immunochromatographic assay device further
comprises a test strip with a plastic housing.

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ABSTRACT

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A method to determine the presence or absence of Streptococcus Group A antigen in a sample, comprising the following steps: extracting the antigen from the sample in an assay chamber with two or less extraction reagents, wherein the two reagents may be added to the assay chamber in no particular sequence; introducing a lateral flow immunochromatographic assay device into the extraction reagents containing the extracted antigen without further addition of reagents or manipulation of the sample; forming an antigen-indicator labeling reagent complex; and determining the presence or absence of the antigen in the sample by the presence or absence of a signal formed by the binding of the antigen-indicator labeling reagent complex to an indicator capture reagent specific for said antigen-indicator labeling reagent complex.

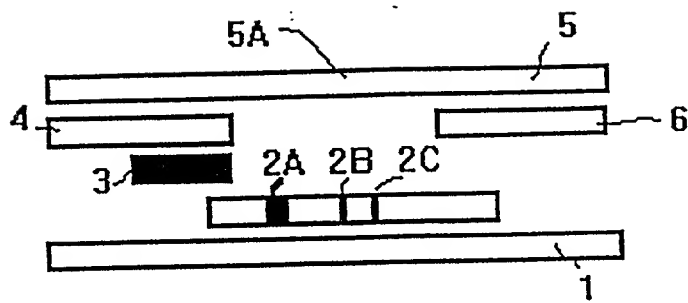


Figure 1

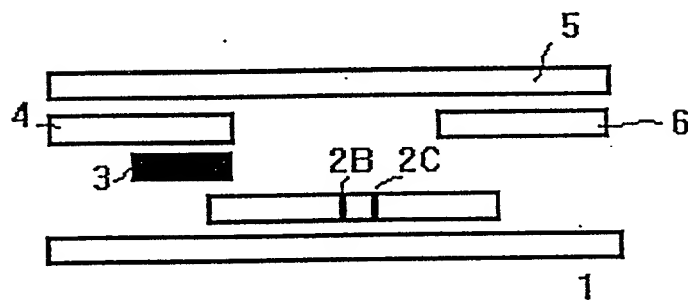


Figure 2

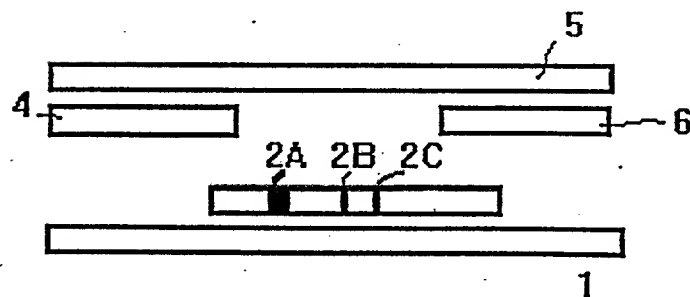


Figure 3

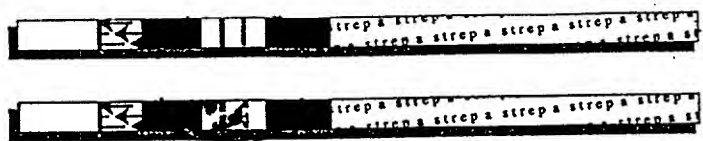


Figure 8(a)

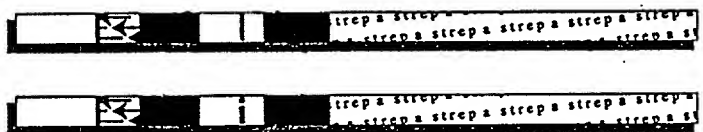


Figure 8(b)

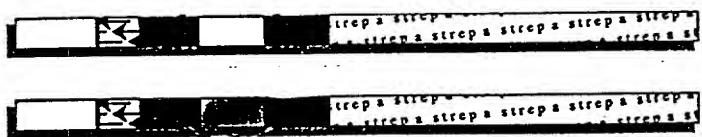


Figure 8(c)

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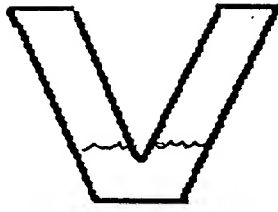


Figure 9(a)

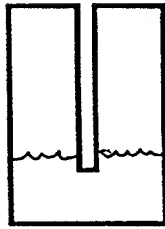


Figure 9(b)



Figure 9(c)

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